89165513

B4

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classificati n 4 : A61K 45/02, 47/00

A1

(11) Internati nal Publication Number: WO 89/ 04177

(43) International Publication Date: 18 May 1989 (18.05.89)

(21) International Application Number: PCT/US88/03883

(22) International Filing Date: 1 November 1988 (01.11.88)

(31) Priority Application Number: 116,434

(32) Priority Date: 3 November 1987 (03.11.87)

(33) Priority Country: US

(71) Applicant: GENENTECH, INC. [US/US]; 460 Point San Bruno Boulevard, South San Francisco, CA 94080 (US).

(72) Inventors: HWANG-FELGNER, Jiin-Yu ; Vical/10955, John J. Hopkins, No. 2, San Diego, CA 92121 (US). JONES, Richard, E. ; 870 Los Robles Avenue, Palo Alto, CA 94306 (US). MAHER, James, F. ; 2503 E. Albany #g, Broken Arrow, OK 74014 (US).

(74) Agents: ADLER, Carolyn, R. et al.; Genentech, Inc., Legal Department, 460 Point San Bruno Boulevard, South San Francisco, CA 94080 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent), DK, FI, FR (European patent), GB (European patent), HU, IT (European patent), JP, KR, LU (European patent), NL (European patent), NO, SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

B

Z

(54) Title: GAMMA INTERFERON FORMULATION

(57) Abstract

A liquid pharmaceutical composition comprising an effective amount of non-lyophilized gamma-interferon. The liquid pharmaceutical composition which additionally includes a buffer capable of maintaining the pH of the liquid composition within the range of 4.0 to 6.0, a stabilizing agent and a non-ionic detergent.

STABLE LIQ. COMPSN. CONTG. NON-LYOPHILISED GAMMA INTERFERON + AN PREF. BUFFER, SUGAR ALCOHOL STABILISER AND NONIONIC DETERGENT

3399

89165513

Gamma Interferon Formulation

Field of the Invention

This invention relates to a stable biologically active gamma-interferon liquid formulation.

Background of the Invention

Immune or gamma-interferon was originally classified on a physical basis as Type II Interferon due to its lability to acid treatment and/or heating to 56°C. This operational classification distinguished it from virus-induced or Type I Interferons (alpha and beta) which, in general, are not acid or heat labile. As a result of the widespread availability of specific antisera against each of the major interferon classes (alpha, beta, and gamma), classification and distinction of each type is now usually made by serological or immunological methods. Despite this, gamma-interferon preparations are still identified as such by their rapid inactivation upon acid treatment. See, The Interferon System, 2nd edition, W.E. Stewart II, Springer-Verlag, New York, 1981.

Gamma-interferon has been employed in clinical studies for many years. The methods currently available for preparing gamma-interferon dosage forms comprises lyophilizing the gamma-interferon in combination with other ingredients for reconstitution with an appropriate diluent at the time of use. Because gamma-interferon is known to be acid labile, it has traditionally been handled at neutral or slightly alkaline pH. See, for example, U.S. Patent No. 4,499,014 which discloses reactivation of a lyophilized acidic gamma-interferon solution to a pH of 6 to 9. U.K. Patent Application GB 2119313A discloses lyophilized formulations of gamma-interferon reconstituted at pH 7.5. Neutral or slightly alkaline solutions of higher concentrations of gamma-interferon are unusable as injectable formulations because of the immediate formation of a visible precipitate. Such precipitates may cause thrombosis n administration or decrease potency. Eur pean Patent

Application Publication No. 0196203 discloses reconstitution of lyophilized gamma-interferon to a pH of 4 to 6.0.

An object of the present invention is to provide a biologically active, stable liquid formulation of gamma-interferon for use in injectable applications. Another object of this invention is to provide a formulation which does not require prior lyophilization of a gamma-interferon composition. It is another object of this invention to prevent dimer and oligomer formation consequent to lyophilization of gamma-interferon. Yet another object of this invention is to provide a liquid formulation containing biologically active gamma-interferon having improved stability. Still another object of this invention is to provide a liquid formulation permitting storage for a long period of time in a liquid state facilitating storage and shipping prior to administration. Still another object of this invention is to reduce aggregation of gamma-interferon, particularly that associated with heating. Another object of this invention is to provide a liquid formulation resistant to fluctuations in temperature. Yet another object of this invention is the elimination from the preparation of a bulking or stabilizing agent such as human serum albumin (HSA). Still another object of this invention is to reduce potential contamination by other proteins and other blood contaminants which may be associated with human serum albumin. Yet another object of this invention is to provid a liquid formulation which is easily made and administered having eliminated lyophilization and reconstitution steps. Yet another object of this invention is to provide a pharmaceutical composition containing non-lyophilized gamma interferon that can be produced less expensively.

Summary of the Invention

The objects of this invention are accomplished by a liquid pharmaceutical compositi n comprising an effective amount of biologically active non-lyophilized gamma-interferon. The liquid

pharmaceutical composition may additionally include a buffer capable of maintaining the pH of the liquid formulation within the range of 4.0 to 6.0, a stabilizing agent and a nonionic detergent. In a preferred embodiment of the liquid formulation of this invention the pH will be in the range of 4.5 to 5.5, preferably at pH 5.0. The gamma-interferon of this invention is not lyophilized but, rather, once prepared from sources using methods known to the ordinarily skilled artisan is included directly in the formulation of this invention. The stabilizing agent of this invention is typically a polyhydric sugar alcohol. It was not appreciated until this invention that a liquid formulation of gamma-interferon could be made which retains biological activity, has a long shelf-life and can be administered therapeutically without lyophilization and reconstitution. In addition, it was not appreciated until this invention that a liquid formulation of gamma-interferon at pH of from 4 to 6 would decrease aggregation, reduce thermal unfolding of the protein and maintain biological activity. It was also not appreciated until this invention that a non-lyophilized liquid formulation at pH 5.0 could have an extended shelf life. Accordingly, the invention is directed to a liquid pharmaceutical composition comprising an effective amount of non-lyophilized gamma interferon for therapeutic administration.

Detailed Description

Gamma interferon and its methods of preparation, including synthesis in recombinant cell culture, are well known (EP 77, 670A and 146, 354A). Included within the scope of gamma-interferon are gamma interferon from recombinant or native sources as well as gamma-interferon variants, such as amino acid sequence variants, e.g., Cys-Tyr-Cys or desCys-Tyr-Cys amino terminal species. Also included are other insertions, substitutions or deletions of one or more amino acid residues, glycosylation variants, unglycosylated gamma-interferons, organic and inorganic salts and covalently modified derivatives of gamma-interferon. The effective amount of gamma-interferon to be formulated in the liquid composition is

selected based on several variables, including the disease to be treated and therapeutic regimen. Generally the gamma-interferon has an activity in a standard bioassay in the range of $1x10^6$ to $2x10^7$ U/mg protein or more.

Examples of the polyhydric sugar alcohols to be used as the stabilizer in the present invention to insure isotonicity of the composition are those of trihydric or higher, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. These polyhydric sugar alcohols can be used alone or in a combination thereof. In view of stabilization of interferon, the sugar alcohol is formulated in an amount of 1% to 25% by weight and preferably, 2% to 5% by weight taking into account the amounts of the other ingredients.

The organic acid buffers to be used in the present invention to maintain the pH in the range of about 4.0 to 6.0 and preferably from 4.5 to 5.5 can be conventional buffers of organic acids and salts thereof such as citrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), succinate buffers (e.g., succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, etc.), tartrate buffers (e.g., tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (e.g., gluconic acid-sodium gluconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium gluconate mixture, etc.), oxalate buffers (e.g., oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalat mixture, etc.), lactate buffers (e.g., lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.) and acetate buffers

(e.g., acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.). It is noteworthy that inorganic acid buffers such as phosphate buffers which have been used traditionally do not maintain the pH of the liquid formulation at the desired pH.

Examples of the non-ionic detergents include such surfactants as pluronics, for example, polysorbate 80 and polysorbate 20. The non-ionic detergent is present in a range of .05 mg/mL with a preferred range of about .07 to .2 mg/mL and a most preferred amount of about 0.1 mg/mL.

The liquid formulation of this invention at a pH of 4 to 6, preferably 4.5 to 5.5 and most preferably at pH 5, demonstrates limited aggregation upon warming. Rather than being labile the liquid formulation of this invention is stable for prolonged periods. The formulation of this invention may be stored in a liquid state at various temperatures. A preferred st rage temperature is in the range of -20°C to 30°C with a most preferred temperature storage range of about between 2° and 8°C. All of the components are important for maintenance of biological activity and physical stability. Furthermore, the liquid formulation of this invention will retain biological activity and physical stability without freezing. This avoids potential aggregation upon thawing.

The following examples illustrate the present invention, but are not to be construed to limit the scope of the invention.

Example 1

Liquid Formulation

Human recombinant gamma-interferon (20 x 10^6 U/mg) was formulated by adding either 1.0 or 0.2 mg/mL to: succinic acid (0.27 mg/mL); disodium succinate (0.73 mg/mL); mannit l (40 mg/mL); polysorbate 20 (0.1 mg/mL); and a sufficient quantity of Water For Injection (USP). This liquid formulation was found to exhibit a

long shelf life when maintained at a storage temperature of about between 2° and 8°C in a liquid state. The succinate buffer maintained the liquid formulation at pH 5.0. The non-ionic detergent prevented aggregation during shipping and handling. The sugar rendered the formulation isotonic without the need for the addition of salts, which have been shown to cause aggregation of gamma-interferon. And further, the sugar appears to stabilize the pharmaceutical composition of this invention (compare the succinate/mannitol lyophilized formulation to the HSA/phosphate lyophilized formulation).

The liquid formulation of this invention using 0.2 mg/mL of non-lyophilized gamma-interferon was compared to two other lyophilized formulations of gamma-interferon. As seen in Table I below, the loss of bioactivity reflected in the rate constants was ten-fold greater for the succinate/mannitol lyophilized formulation and five-fold greater for HSA/phosphate lyophilized formulation than the liquid formulation of this invention. These changes in the bioactivity are reflected in the rate constant which is the slope of the line resulting from a plot of the natural logarithm of the loss of bioactivity of the gamma-interferon formulation versus time. Bioactivity was measured using a viral protection assay known to the ordinarily skilled artisan. The lyophilized compositions were stored in lyophilized form and were reconstituted at various times to determine the bioactivity remaining in the lyophilized preparation. The shelf life of the liquid formulation of this invention was considerably greater than that of the lyophilized formulations. The greater shelf life of the liquid formulation relative to the lyophilized formulation listed in Tabl 1 shows that the liquid formulation of this invention retains biological activity ten times longer than the lyophilized compositions.

Table 1

Comparative Stability of Gamma-Interferon Formulated at 0.2 mg/mL[1]

Formulation	Study (months)	Rate Constant X 10^{-3}	Relative Shelf Life (days)[2]
Succinate/ Mannitol Lyophilized	6	2.854	1
Succinate/ Mannitol Liquid	4	0.205	10
HSA/ Phosphate Lyophilized[3]	3	1.038	5

[1] Based on real time 5°C data.

[2] A comparison of the relative stability based on the bioactivity of the three formulations with the succinate/mannitol lyophilized composition being arbitrarily set at 1.

[3] This formulation was prepared by mixing 0.20 mg lyophilized gamma-interferon, 10 mg HSA, 5 mM sodium phosphate pH 7.0 and reconstituted with 0.9% saline.

A similar comparative study was carried out for the liquid formulation of this invention using 1.0 mg/mL of non-lyophilized human recombinant gamma-interferon. Once again, as shown in Table 2, the loss of bioactivity was greater for the lyophilized formulation than for the liquid formulation of this inventi n. Table 2 also shows that the shelf life of the liquid formulation of this invention was three times greater than that of the lyophilized formulation.

Table 2

Comparative Stability of Gamma-Interferon Formulated at 1.0 mg/mL[1]

Formulation	Study Time (months)	Rate Constant X 10^{-3}	Relative Shelf Life (days)[2]
Succinate/ Mannitol Lyophilized	14	0.485	1
Succinate/ Mannitol Liquid	14	0.179	3

[1] Based on real time 5°C data.

[2] A comparison of the relative stability based on the bioactivity of the two formulations with the succinate/mannitol lyophilized composition being arbitrarily set at 1.

Claims:

1. A liquid pharmaceutical composition comprising an effective amount of non-lyophilized gamma-interferon.

2. A liquid pharmaceutical of claim 1 which additionally includes a buffer capable of maintaining the pH of the liquid composition within the range of 4.0 to 6.0, a stabilizing agent and a non-ionic detergent.

3. A liquid pharmaceutical composition of claim 2 wherein the buffer is an organic acid buffer.

4. A liquid pharmaceutical composition of claim 3 wherein the organic acid buffer is selected from the group consisting f citrate, succinate, tartrate, fumarate, gluconate, oxalate, lactate and acetate.

5. A liquid pharmaceutical composition of claim 2 wherein the stabilizing agent is a polyhydric sugar alcohol.

6. A liquid pharmaceutical composition of claim 5 wherein the polyhydric sugar alcohol is selected from the group consisting of glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

7. A liquid pharmaceutical composition of claim 6 wherein the sugar alcohol is added in an amount of about 1% to 25% by weight based on the composition.

8. A liquid pharmaceutical composition of claim 6 wherein the sugar alcohol is added in an amount of about 2% to 5% by weight based on the composition.

9. A liquid pharmaceutical composition of claim 2 wherein the non-ionic detergent is selected from the group consisting of polysorbate 20 and polysorbate 80.

10. A liquid pharmaceutical composition of claim 2 wherein the pH of the liquid composition is in the range of 4.5 to 5.5.

11. A liquid pharmaceutical composition of claim 2 wherein the pH of the liquid composition is at a pH of 5.0.

12. A liquid pharmaceutical composition of claim 2 which is sterile.

13. A liquid pharmaceutical composition of claim 2 which is isotonic to blood.

14. A liquid pharmaceutical composition of claim 1 that is stored for more than two weeks and then administered therapeutically.

15. A method of treatment of a disease using gamma interferon comprising administration of a liquid pharmaceutical composition comprising an effective amount of non-lyophilized gamma-interferon.

16. The method of treatment of claim 15 wherein the liquid pharmaceutical composition additionally includes a buffer capable of maintaining the pH of the liquid composition within the range of 4.0 to 6.0, a stabilizing agent and a non-ionic detergent.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/03883

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 45/02; A 61 K 47/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Patent Abstracts of Japan, vol. 9, no. 28 (C-264)(1751), 6th February 1985 & JP, A, 59175416 (SUNSTAR K.K.) 4 November 1984	1
Y	--	2-14
X	Patent Abstracts of Japan, vol. 11, no. 139 (C-420)(2586), 7th May 1987 & JP, A, 61277633 (TORAY IND. INC.) 8 December 1986	1
Y	--	2-14
A	EP, A, 0080879 (SUNSTAR K.K et al.) 8 June 1983, see page 2, line 10 - page 5, line 13; page 7, line 12 - page 12, line 3	
P,X	-- EP, A, 0258683 (Dr KARL THOMAE) 9 March 1988, see the whole document	1

89165513

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17th February 1989	17. 03. 89

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

Form PCT/ISA/210 (second sheet) (January 1985)

3411

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers XX because they relate to subject matter not required to be searched by this Authority, namely:

XX Claims 15, 16
See PCT Rule 39.1(iv)
Methods for treatment of the human or animal body by means of surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers ..... because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8803883 SA 25361

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 13/03/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0080879	08-06-83	JP-A- 58092619	02-06-83
		US-A- 4675184	23-06-87
		JP-A- 58092620	02-06-83
		JP-A- 58092621	02-06-83
		JP-A- 58092622	02-06-83
		JP-A- 58167520	03-10-83
EP-A- 0258683	09-03-88	AU-A- 7730787	25-02-88
		DE-A- 3628468	03-03-88
		JP-A- 63051338	04-03-88

89165513

3413

EPO FORM 1503

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82